Citation for the original published paper (version of record):
Relative importance of column and adsorption parameters on the productivity in preparative liquid chromatography II: Investigation of separation systems with competitive Langmuir adsorption isotherms. P. Forssén, J. Samuelsson, T. Fornstedt. J. Chromatogr. A. 1347 (2014) 72–79.

doi:10.1016/j.chroma.2014.04.059

Relative Importance of Column and Adsorption Parameters on the Productivity in Preparative Liquid Chromatography II: Investigation of Separation Systems with Competitive Langmuir Adsorption Isotherms

Patrik Forssén[1,2], Jörgen Samuelsson[2] and Torgny Fornstedt[1,2]*

[1]Analytical Chemistry, Department of Chemistry - BMC, Uppsala University, P.O. Box 599, SE-751 24, Uppsala, Sweden

[2]Department of Engineering and Chemical Sciences, Karlstad University, SE-651 88 Karlstad, Sweden

*Corresponding author information:

Torgny Fornstedt, Professor, Karlstad University, SE-651 88 Karlstad, Sweden

Torgny.Fornstedt@kau.se, +46 54 700 1960; + 46 76 774 31 58

Abstract

In this study we investigated how the maximum productivity for commonly used, realistic separation system with a competitive Langmuir adsorption isotherm is affected by changes in column length, packing particle size, mobile phase viscosity, maximum allowed column pressure, column efficiency, sample concentration/solubility, selectivity, monolayer saturation capacity and retention factor of the first eluting compound. The study was performed by generating 1 000 random separation systems whose optimal injection volume was determined, i.e., the injection volume that gives the largest achievable productivity. The relative changes in largest achievable productivity when one of the parameters above changes was then studied for each system and the productivity changes for all systems were presented as distributions.

We found that it is almost always beneficial to use shorter columns with high pressure drops over the column and that the selectivity should be greater than 2. However, the sample concentration and column efficiency have very limited effect on the maximum productivity. The effect of packing particle size depends on the flow rate limiting factor. If the pumps maximum flow rate is the limiting factor use smaller packing, but if the pressure of the system is the limiting factor use larger packing up to about 40 µm.

Keywords: preparative chromatography; process optimization; numerical optimization; Monte Carlo simulations; productivity

1 Introduction

Modern computers and refined simulation algorithms [1–4] makes it possible to perform computer-assisted optimization of preparative chromatography on ordinary PC's. The optimization criteria often used is the maximum productivity, possibly with a yield constraint, as the cost of operating the separation system and the value of the separated compounds are seldom known.

By using computer-assisted optimization we have, in a previous study [5], investigated how the maximum productivity, for a single specific chiral system, depends on changes in column length, packing particle size, maximum column pressure, column efficiency, sample concentration/solubility, selectivity, retention factor of the first eluting compound and monolayer saturation capacity. We found that the parameters that affected the productivity the most were the selectivity, the retention factor of the first eluting compound and the column length. However, the column efficiency and sample concentration/solubility were found to be of minor importance.

Our previous study [5] above might give some indication of the relative importance of the different parameters, however, we cannot draw any more general conclusions from just studying a single case. In this study we want a more general picture and therefore use Monte Carlo simulations where we study 1 000 randomly selected separation systems. The random systems are here limited to the commonly used realistic ones where the adsorption of the solutes are described using the competitive Langmuir adsorption isotherm. To better mimic a realistic situation we have also introduced pressure and flow rate constrains on the systems. We will then examine, for each randomly selected system, how the maximum productivity is affected by changes in column length, packing particle size, maximum allowed column pressure, column efficiency, sample concentration/solubility, mobile phase viscosity, selectivity, monolayer saturation capacity and retention factor of the first eluting compound.

It should be noted that the primary aim of this study, and our previous one [5], is *not* to try to answer the question: which column length, packing particle size etc. will give the optimal productivity? Instead the aim is to examine how *changes* in one of studied parameters will affect the maximum achievable productivity. The goal of this study is to determine which of the studied parameters that are most likely to affect the maximum productivity of the separation process the most. Here it should be noted that parameters that have major effect on the productivity are also more likely to affect the reproducibility of the separation process.

This study also intends to provide a more general guidelines than the previous study [5] for column selection/design and this information can be important both for users and manufacturers of preparative separation systems.

2 Theory

Here we will limit our study to separation systems with the following properties,

1) The columns used are packed with small, almost spherical non-compressible particles (diameter less than 50 µm).
2) The flow through the column is laminar and the flow velocity can be related to the inlet pressure by the Darcy’s law, this is almost always the case in HPLC [6].
3) The adsorption mass transfer resistance is small; this is typically the case for small molecules with moderate polarity [6].

4) The dispersion coefficients dependence on mobile phase velocity can be described using the Knox plate height equation; this equation has been shown to usually fit experimental data well [7].

5) The adsorption can be described using the competitive Langmuir adsorption isotherm. This adsorption isotherm assumed that we have one type of adsorption site, the adsorption occurs in a single layer and adsorbate/adsorbate interactions are negligible [1].

When the adsorption mass transfer resistance is small the Equilibrium-Dispersive column model [1] can be used to accurately model elution profiles. Here we will therefore use this model and a finite volumes solver [8,9], with rectangular injection profiles as boundary conditions, to calculate elution profiles.

We will always use the maximum achievable flow rate, $F_{\mathrm{V,max}}$, trough the column. The maximum flow rate is restricted by either the pump's maximum flow rate ($F_{\mathrm{V,max,pump}}$) or the maximum allowed pressure drop (ΔP_{max}) over the system. Using Darcy's law this can be calculate according to,

$$\begin{gathered} F_{\mathrm{V,max}} = \min\left(k_{\mathrm{flow}} \frac{\Delta P_{\mathrm{max}} d_{\mathrm{p}}^2 D^2}{\eta L},\ F_{\mathrm{V,max,pump}} \right) \\ \text{where } \Delta P_{\mathrm{max}} = \min\left(\Delta P_{\mathrm{max,pump}},\ \Delta P_{\mathrm{max,col}} \right),\ k_{\mathrm{flow}} = \frac{\theta \varepsilon \pi}{4}, \end{gathered} \tag{1}$$

here $\Delta P_{\mathrm{max,pump}}$ is pump max working pressure, $\Delta P_{\mathrm{max,col}}$ is column pressure rating, θ is a column structural constant, ε is the column porosity, d_{p} is the particle size, D is the column diameter, η is the mobile phase viscosity and L is the column length. The column pressure drop, ΔP_{col}, and the mobile phase linear velocity, u, that corresponds to the maximum flow rate, $F_{\mathrm{V,max}}$, can be calculated using the Darcy's law,

$$\Delta P_{\mathrm{col}} = \frac{F_{\mathrm{V,max}} \eta L}{k_{\mathrm{flow}} d_{\mathrm{p}}^2 D^2},\ u = \frac{F_{\mathrm{V,max}}}{\varepsilon D^2 \pi / 4}. \tag{2}$$

We will use the Knox plate height equation to calculate the column efficiency, i.e., the number of theoretical plates, N_{x}. According to this equation the reduced plate height, h, is,

$$h = 2\big/\nu + \nu^{1/3} + \nu\big/10\,,\quad \text{where } \nu = \frac{u d_\mathrm{p}}{D_\mathrm{m}},\ H = h d_\mathrm{p}, \tag{3}$$

here H is the actual plate height and D_m is a diffusion constant that according to Stokes-Einstein's equation $\propto 1/\eta$. We then have that,

$$N_\mathrm{x} = \frac{L}{H} = \frac{L}{h d_\mathrm{p}}. \tag{4}$$

The adsorption of the two compounds, where compound 1 is the first eluting one and compound 2 is the later eluting one, can be described using the competitive Langmuir adsorption isotherm model if we have one type of adsorption site, the adsorption occurs in a single layer and adsorbate/adsorbate interactions are negligible. Here we have that for compound *i* (1 or 2),

$$q_i = \frac{a_i C_i}{1 + \sum_{j=1}^{2} b_j C_j}, \tag{5}$$

where q is the stationary phase concentration, C is the mobile phase concentration and a, b are adsorption isotherm parameters. The saturation capacity for compound *i*, $q_{\mathrm{s},i}$, is defined to be,

$$q_{\mathrm{s},i} = \lim_{C_{j \neq i} = 0,\, C_i \to \infty} q_i = \frac{a_i}{b_i}, \tag{6}$$

the retention factor for compound *i*, k_i, is defined to be,

$$k_i = \frac{1-\varepsilon}{\varepsilon} \cdot \left.\frac{\partial q_i}{\partial C_i}\right|_{C_{*}=0} = \frac{1-\varepsilon}{\varepsilon} a_i, \tag{7}$$

and the selectivity α between compound 1 and 2, is defined to be,

$$\alpha = \frac{k_2}{k_1} = \frac{a_2}{a_1}. \tag{8}$$

Given the saturation capacity, $q_{\mathrm{s},i}$, for both compounds, the retention factor for the first compound, k_1, and the selectivity α the adsorption isotherm parameters can be calculated according to,

$$a_1 = \frac{\varepsilon}{1-\varepsilon} k_1,\; a_2 = \alpha \frac{\varepsilon}{1-\varepsilon} k_1,$$
$$b_1 = \frac{\varepsilon}{1-\varepsilon} \cdot \frac{k_1}{q_{s,1}},\; b_2 = \alpha \frac{\varepsilon}{1-\varepsilon} \cdot \frac{k_1}{q_{s,2}}. \tag{8}$$

The process optimization will be performed using the maximum allowed flow rate, see Eq. (1), and the injection volume that maximizes the productivity will be determined. Here the productivity, P_R, is normalized with respect to the column volume, $V_{col,}$ i.e., for compound *i* we have that,

$$P_{R,i} = \frac{n_{coll,i}}{t_c V_{col}} \text{ where } n_{coll,i} = F_V \int_{t_{start,i}}^{t_{stop,i}} C_i(t)\,dt,\; V_{col} = \frac{\pi D^2 L}{4}, \tag{9}$$

where n_{coll} is the amount collected between the fractional cut point times t_{start} and t_{stop}, t_c is the cycle time, i.e., the time elapsed between when the first injected compound begins to elute and when the last compound is completely eluted. Here the cycle is assumed to begin when then concentration sum of the eluted compounds first is greater than 1/100 of the maximum concentration sum achieved and ends the last time it is less than 1/100 of it. The yield, *Y*, of compound *i* is defined to be,

$$Y_i = \frac{n_{coll,i}}{n_{inj,i}}, \tag{10}$$

where $n_{inj,i}$ is the injected amount the compound, i.e., the yield gives how much of the injected amount is collected. Here we will use the yield requirement $Y_i \geq Y_{min}$ in the process optimization, i.e., it is required that the yield is equal to or larger than a set, fixed limit Y_{min}. Note that this does *not* mean that the achieved yield in the process optimization will be equal to the set limit Y_{min}, only that it cannot be lower. The purity, *PU*, for compound *i* is defined to be,

$$PU_i = \frac{n_{coll,i}}{F_V \sum_j \int_{t_{start,i}}^{t_{stop,i}} C_j(t)\,dt}, \tag{11}$$

i.e., the fraction collected of compound *i* of the total collected amount of all compounds between the fractional cut point times t_{start} and t_{stop}. Here we will also use a purity requirement that $PU_i \geq PU_{min}$ in the process optimization, i.e., it is required that the purity is equal to or larger than the set limit PU_{min}.

3 Calculations

3.1 Random Systems

1 000 realistic random systems were generated with parameters set according to Table 1. For parameters with intervals in the table, the parameter for a specific system was set using a uniformly distributed random value in that interval. The maximum flow rate $F_{\mathrm{V,max}}$, maximum column pressure drop ΔP_{max}, linear velocity u and number of theoretical plates for the first eluting compound $N_{\mathrm{x},1}$ are calculated using the parameters in Table 1 and Eq. (1), Eq. (2), Eq. (4) and will be within the ranges given in Table 2.

The values in Table 1 are based on literature and information from manufacturers. The mobile phase viscosity, η, range represents eluents ranging from heptane to isopropanol. Maximum column length, L, and column pressure rating, $\Delta P_{\mathrm{max,col}}$, are inspired by the novasep Prochrom DAC columns. Pump max working pressure, $\Delta P_{\mathrm{max,pump}}$, are inspired by the novasep Prochrom Hipersep product line. The minimum column length, L, was set according to personal communication with Johan Högblom at AkzoNobel, the manufacturer of Kromasil, according to them using preparative columns shorter than 10 cm makes it more difficult to achieve reproducible results. The diffusion constant range for the first compound, $D_{\mathrm{m},1}$, is set so that the corresponding theoretical plates range will cover a wide range of different molecules, see Table 2, and the theoretical plate difference between the compounds was inspired by on our previous experience [10].

For a generated random system to be accepted it was checked that a small 1 µL injection gave a separation that fulfilled the system's required minimum yield and required minimum purity, see Eq. (10) and Eq. (11). The reason for rejection of random system that did not fulfil these requirements is that we want to study the relative change in largest achievable productivity when a system- or adsorption parameter is changed. If the random system does not fulfil the requirements the maximum achievable productivity is 0 and will result in division by zero when calculating the relative changes in largest achievable productivity, see Eq. (13).

3.2 Process Optimization

For a random system we want to determine the injection volume that gives the largest productivity, $P_{\mathrm{R,max}}$, subject to the yield and purity constraints, this is an optimization problem than can be written,

$$\begin{cases} \max\limits_{V_{\mathrm{inj}}} P_{\mathrm{R}}\left(V_{\mathrm{inj}}\right), \\ \text{such that} \\ Y \geq Y_{\min}, \\ PU \geq PU_{\min}, \end{cases} \tag{12}$$

see also Eq. (9)-(11), and this optimization problem will solved using a Nelder–Mead simplex method [11]. Note that each random system have a single fixed value for the system- and adsorption parameters that are intervals in Table 1 and this value is randomly selected in the given interval, i.e., these values are *not* allowed to vary.

Here we want to study relative changes in largest achievable productivity when we make small relative changes in the system- or adsorption parameters. To study this one would ideally like to estimate the derivative of $P_{\mathrm{R,opt}}$ with respect to the studied parameter. For example, if the largest achievable productivity for a random system with column length L_0 is $P_{\mathrm{R,opt}}(L_0)$ we would like to estimate,

$$\begin{gathered} \left.\frac{d\left(\Delta P_{\mathrm{R,opt}}\right)}{d\left(\Delta L\right)}\right|_{L=L_0} = \lim_{L_1 \to L_0} \frac{\Delta P_{\mathrm{R,opt}}\left(L_0, L_1\right)}{\Delta L\left(L_0, L_1\right)}, \text{ where,} \\ \Delta P_{\mathrm{R,opt}}\left(L_0, L_1\right) = \frac{P_{\mathrm{R,opt}}\left(L_1\right) - P_{\mathrm{R,opt}}\left(L_0\right)}{P_{\mathrm{R,opt}}\left(L_0\right)}, \ \Delta L\left(L_0, L_1\right) = \frac{L_1 - L_0}{L_0}, \end{gathered} \tag{13}$$

here $\Delta L(L_0, L_1)$, $\Delta P_{\mathrm{R,opt}}(L_0, L_1)$ is the relative change in column length and the largest achievable productivity, respectively, when the column length is changed from L_0 to L_1. $P_{\mathrm{R,opt}}$ must be calculated using a computer algorithm that estimates the solution to the Equilibrium-Dispersive column model in the optimization problem Eq. (12). $P_{\mathrm{R,opt}}$ will therefore contain some numerical noise that makes it impossible to numerically estimate the derivative in Eq. (13) as usual by setting $L = L_0 + \varepsilon$, for some small number ε, and then setting,

$$\left.\frac{d\left(\Delta P_{\mathrm{R,opt}}\right)}{d\left(\Delta L\right)}\right|_{L=L_0} \approx \frac{\Delta P_{\mathrm{R,opt}}\left(L_0, L_0+\varepsilon\right)}{\Delta L\left(L_0, L_0+\varepsilon\right)}, \tag{14}$$

because $\Delta P_{\mathrm{R,opt}}(L_0,\ L_0+\varepsilon)$ cannot be estimated accurately due to the numerical noise. We will therefore instead estimate $\Delta P_{\mathrm{R,opt}}(L_0, L_1)$ when $\Delta L(L_0, L_1)$ = 10% in this study (and also use 10% for the relative changes in all other system- or adsorption parameters). Note that if $\Delta P_{\mathrm{R,opt}}(L_0, L_1)$ can accurately be estimated by a linear function of $\Delta L(L_0, L_1)$, i.e., $\Delta P_{\mathrm{R,opt}}(L_0, L_1) = k \cdot \Delta L(L_0, L_1)$ for some parameter k, when $\Delta L \leq 10\%$ then the parameter $\Delta P_{\mathrm{R,opt}}(L_0, L_1)/\ \Delta L(L_0, L_1) = k$ is an accurate estimate of the derivative $\left.d\left(\Delta P_{\mathrm{R,opt}}\right)\big/d\left(\Delta L\right)\right|_{L=L_0}$ in Eq. (13), see also Fig. 2a in [5].

To be consistent we will change each parameter 10%, one at the time, from its initial value in the direction that is most likely to increase the maximum achievable productivity. However, note that direction of change will have small impact on the estimated absolute value of $\Delta P_{\mathrm{R,opt}}$ if $\Delta P_{\mathrm{R,opt}}$ can be accurately estimated by a linear function, or more generally if we have that $\Delta P_{\mathrm{R,opt}}(-p) \approx \pm\Delta P_{\mathrm{R,opt}}(p)$ for the studied parameter p, when the relative parameter change is ≤ 10%. Changing in the direction that is most likely to increase the maximum achievable productivity means that the retention factor for the first eluting compound k_1, the mobile phase viscosity η and the column length L are decreased 10% and the other parameters are increased 10%. For the parameters saturation capacity q_s and sample concentration C_{samp} we will increase those 10% for both compounds.

Notice that a change in the independent parameters column pressure drop ΔP_{col}, viscosity η, particle size d_p or column length L might lead to a change in the dependent parameter maximum allowed flow rate $F_{\mathrm{V,max}}$, see Eq. (1), and any changes in the flow rate will affect the dependent parameter efficiency N_x, see Eq. (3) and Eq. (4). Also notice that a change in a parameter might lead to a system cannot fulfill the required minimum yield Y and required minimum purity PU, see Eq. (12), for any injection volume.

We will always use the maximum allowed flow rate $F_{V,max}$, see Eq. (1), and only consider the injection volume in the process optimizations. In total 18 000 process optimizations will be performed in this study. In order to have realistic runtimes the process optimizations was therefore performed in parallel on a computer cluster with, in total, 16 physical calculation cores.

4 Result and Discussion

First we must considered if 1 000 random systems are an adequate "sample size" or if we need to have more systems. One way to study this is to examine the accuracy of some properties of the optimal productivity distribution, e.g. how the accuracy of mean, median, standard deviation etc. varies with the number of studied random systems.

In Fig. 1 the width of the 95% confidence interval for the median of the optimal productivity, $P_{R,opt}$, distribution changes for the first eluting compound, when the maximum column pressure drop ΔP_{max} increases 10%, is plotted against the number of random systems. As can be seen the width of the confidence interval does not significant decrease by considering more random systems than 1 000. We observed the same trend for all other studied parameter and could therefore conclude that the number of studied random systems is adequate.

4.1 Flow Rate Limited by Maximum Column Pressure

In this part we will assume that the flow rate is only limited by the columns pressure rating $\Delta P_{max,col}$. This means that an increase in the maximum column pressure drop ΔP_{max} involves changing to a column that can handle a higher maximal pressure, i.e., have a higher $\Delta P_{max,col}$ value.

We begin by studying a histogram showing how the optimal productivity $P_{\mathrm{R,opt}}$ changes when the particle size is decreased 10%, see Fig. 2. As can be seen the histogram is heavily skewed and we cannot assume that the results can be modeled with standard normal distributions. One way of processing the result is to instead to consider the generalized hyperbolic distribution [12] that can accurately describe this type of heavily skewed distributions. The parameters in the generalized hyperbolic distribution were estimated by a maximum likelihood procedure and in Fig. 2 we can see that the Generalized Hyperbolic distribution describe the observed data well. The bar above the histogram shows the 2.5 – 97.5 percentile range of the generalized hyperbolic distribution, i.e., 95% of the distribution will lay in this interval, with the median marked as a cross.

In Fig. 3 the bars show the 2.5 – 97.5 percentile range of the generalized hyperbolic distribution for all parameter distributions, i.e., 95% of the distribution will lay in this interval, with the median marked as a cross, cf. Fig. 2. As can be seen from the median values in Fig. 3a decrease in the column length L or an increase in particle size d_{p} will lead to the largest relative increases in the optimal productivity; also an increase in maximum column pressure drop ΔP_{max} or a decrease in mobile phase viscosity η will lead to significant relative increases in the optimal productivity. All these parameters affect the maximum allowed flow rate $F_{\mathrm{V,max}}$ according to Eq. (1), changing these parameter will mean an increase in $F_{\mathrm{V,max}}$ and this is usually advantageous as this will lead to shorter cycle times and hence increased optimal productivity, see Eq. (9). This is interesting because these parameters are not strictly dependent on a specific separation problem, except maybe the viscosity η that can often be lowered by increasing the temperature. Changing the separation dependent adsorption parameters saturation capacity q_{s}, selectivity α and retention factor for the first elution compound k_1, will have roughly the same effect on the optimal productivity. Finally changes in the efficiency N_{x} and the sample concentration C_{samp} will both have a very limited effect on the optimal productivity. Surprisingly, no large difference is observed in Fig. 3 when the target compound is the first or second eluting compound; the only difference is that the selectivity α are slightly more important for the last eluting compound.

In Fig. 4 we have plotted 1 – the cumulative distribution function of the estimated generalized hyperbolic distribution, i.e., the figure shows the probability that a change in a parameter will lead to a relative change in the optimal productivity that exceeds a certain value. From this figure we see a decrease in the column length L or an increase in particle size d_p are most likely to lead to large relative increases in the optimal productivity. For example, there is much higher probability that the relative change in optimal productivity change exceeds 15% for changes in column length L or an increase in particle size d_p than for changes in the other parameters; however, increasing the efficiency N_x and selectivity α can also in some cases lead to very large productivity gains whereas the productivity gains for changes in the rest of the parameters very rarely exceeds 10%. Inspecting the probability that the relative change in maximum productivity $P_{R,opt}$ exceeds 0%, i.e., the y-axis, we see that increasing the saturation capacity q_s and efficiency N_x is the only changes that always will lead to a positive change in the maximum productivity while increasing the particle size have only ≈ 80% probability to give a positive increase in the maximum productivity. Changes in the other parameters will have ≈ 90 - 95% probability to increase the maximum productivity.

Three parameters stand out in Fig. 3 and Fig. 4, the selectivity α and the efficiency N_x where we have a very large percentile range and the particle size d_p where there is a relatively large probability that an increase will lead to a decrease in the optimal productivity. This warrants closer investigation and in Fig. 5 we have plotted how the changes in relative optimal productivity depends on from which initial value the particle size d_p, the efficiency N_x and the selectivity α are increased. As can be seen from Fig. 5(a) if the particle size is greater than ≈ 40 µm for the first eluting compound, or greater than ≈ 45 µm for the last eluting compound, a further increase in particle size are likely to lead to a decrease in the optimal productivity for that compound. Kaczmarski and Antos also found that optimal particle size for later eluting compounds is generally larger than those of the earlier eluting compounds [13]. The reason for this is that for very large particles we will have a very large maximum flow rate $F_{V,max}$ and here we always use the maximum flow rate in the process optimization. Although a increasing the flow rate is usually always advantageous there is an upper limit where it becomes disadvantageous to increase it further. Above this upper limit the decrease in separation of the compounds with higher flow rate will have larger effect on the optimal productivity than the shortened cycle time, see Eq. (9). In Fig 5(b) we see how an increase in the efficiency N_x will have a major impact on the optimal productivity for very low efficiencies, up to around 100 plates, but a minor impact on the productivity for larger efficiencies. This is maybe not surprising because the shape of overloaded elution profiles are mainly determined by the adsorption isotherm if the efficiency is not extremely low according to the Shirazi number [1,14]. In Fig. 5(c) we see how an increase in selectivity α will have a very large effect on the optimal productivity if α is less than ≈ 2, but relatively small effect if it's greater than 2, i.e., it is very important to make sure that there is enough separation of the compounds.

4.2 Flow Rate Mainly Limited by Pump Capacity

As can be seen from Table 2 the upper limit for the maximum flow rate $F_{V,max}$ is 300.2 L/min for the random systems in the Table 1, however the 90th percentile is ≈19.1 L/min (i.e. for 90% of the random systems the maximum flow rate will be less than 19.1 L/min). The previous section indicates that it advantageous to use as short column as possible, in our case 10 cm, and have particle size ≈ 40 µm for the first eluting compound. If the column pressure rating, $\Delta P_{max,col}$ is 60 bar and the mobile phase viscosity η is 1.5 cP then the maximum flow rate $F_{V,max}$ will be ≈ 23 L/min.

The flow rates above are unrealistic to achieve with a normal preparative pump, we will therefore consider what happens if we limit the pump's max flow $F_{V,max}$ to a realistic value rather than assuming that it is unlimited as in the previous section. A realistic preparative pump can for example deliver a max flow of 2.4 L/min and we will use this value for $F_{V,max}$, and use the same values and intervals for all other parameters in Table 1, and generate 1 000 new random systems that we optimize as in the section above. Notice that now the pump's max flow rate can limit the maximum flow $F_{V,max}$ through the column, not only the column pressure rating $\Delta P_{max,col}$ as in the previous section, see Eq. (1).

In Fig. 6 we have plotted histograms and estimated generalized hyperbolic distributions (black lines) of the optimal productivity changes when the maxim column pressure drop $\Delta P_{\max}$ is increased 10%, the column length L is decreased 10%, the particle size d_{p} is increased 10% and the mobile phase viscosity η is decreased 10%; changes in the other parameters (not shown) had almost the same effect on the optimal productivity as in the previous section. Notice that for changes in L, d_{p} and η we will now have bimodal probability density distributions for the changes in optimal productivity. The grey lines in Fig. 6 shows the estimated generalized hyperbolic distributions without pump flow restrictions presented in the previous Section see Fig. 3.

In Fig. 6(a) we see that an increase in the maximum column pressure drop $\Delta P_{\max}$ will have almost the same effect on the optimal productivity as in the previous section, but now the increase usually corresponds to getting a pump that can deliver higher flow rate rather than getting a column that can operate at higher pressure.

In Fig. 6(b) we have a bimodal probability density distribution for decreases in the column length L where the part on the left corresponds to systems where the maximum flow through the column is limited by the pump's capacity and the part to the right corresponds to systems where the maximum flow through the column is instead limited by the column pressure rating $\Delta P_{\max,\mathrm{col}}$. This can easily be observed from the maximum of the probability density distribution for systems without pump flow restrictions (grey line) that coincides with the right distribution. However, for all systems it is usually advantageous to use shorter columns, though much more advantageous if the maximum flow through the column is limited by the column pressure rating. For systems whose maximum flow through the column is limited by the pump's capacity shortening the column will not lead to a higher flow through the column, but the sample will still spend shorter time inside the column, under preparative non-linear conditions this will reduce the band broadening at the outlet and hence reduce the cycle time which will lead to higher optimal productivity, see Eq. (9).

Also in Fig. 6(c) we have a bimodal probability density distribution for increases in the particle size d_p and the parts corresponds to what limits the maximum flow through the column in the same way as in Fig. 6(b). Notice that for systems whose maximum flow through the column is limited by the pump's capacity (left distribution) it is disadvantageous to increase the particle size and one should instead *decrease* it. For these systems we will not get a higher flow through the column by increasing the particle size, the only effect is that the efficiency decreases, see Eq. (3), which will lead to poorer separation of the compounds.

Finally, for Fig. 6(d), the bimodal probability density distribution parts correspond to what limits the maximum flow through the column in the same way as in Fig. 6(b). Here we see that decreases in the mobile phase viscosity η will have only a mild effect if the systems maximum flow through the column is limited by the pump's capacity (left distribution). The mild increase is due to that the efficiency increases slightly because the diffusion constant is increasing due to Stokes-Einstein's equation, see Eq. (3) and Eq. (4).

5 Conclusions

We investigated 1 000 random separation systems and by using computer simulations studied how the maximum productivity depended on changes in column length, packing particle size, column efficiency, column back pressure, mobile phase viscosity, sample concentration/solubility, selectivity, retention factor of the first eluting compound and monolayer saturation capacity. Note that we do not try to find optimum values of the considered system- and adsorption parameters for the studied random system, instead we investigate how changed in these parameters affect the largest achievable productivity.

The following general guidelines can be given,

- It is almost always advantageous to use as short column as possible. Using a shorter column will however have much larger effect on the maximum productivity if it is the column pressure rating $\Delta P_{\mathrm{max,col}}$ that limits the maximum flow rate rather than the pumps max flow $F_{\mathrm{V,max,pump}}$.
- Increasing the pressure drop ΔP over the column will almost always be advantageous and have a large effect on the optimal productivity. Notice that for system where it is the column pressure rating $\Delta P_{\mathrm{max,col}}$ that limits the maximum flow this corresponds to getting a column with a higher pressure rating, but for systems where the maximum flow is limited by the pumps maximum flow $F_{\mathrm{V,max,pump}}$ this corresponds to getting a pump with a higher maximum flow.
- Decreasing the mobile phase viscosity will have large effect on the maximum productivity **if** it is the column pressure rating $\Delta P_{\mathrm{max,col}}$ that limits the achievable flow. However, if it is the pumps max flow $F_{\mathrm{V,max,pump}}$ that limits the achievable flow a decrease in mobile phase viscosity will have almost no effect.
- The particle size should be selected depending on if it is the column pressure rating $\Delta P_{\mathrm{max,col}}$ or the pump's max flow $F_{\mathrm{V,max,pump}}$ that limits the achievable flow. If it is the column pressure rating $\Delta P_{\mathrm{max,col}}$ one should have ≈40 µm particles but if it the pump's max flow $F_{\mathrm{V,max,pump}}$ one should have as small particles as possible, i.e., decrease the particle size but not so much that you have to also decrease flow rate through the column, see Eq. (1).
- The selectivity α should be greater than 2, but increasing it further will only give a slight increase in the maximum productivity. These parameters were the only one with clear difference between first and second eluting compound. The selectivity is in general more important for the last eluting compound than for the first eluting.
- The saturation capacity q_{s} and the retention factor for the first eluting compound k_1 have moderate effect on the maximum productivity. But increased q_{s} and decreased k_1 will lead to higher productivity.
- Increases in the efficiency N_{x} and the sample concentrations C_{samp} have very limited positive effect on the maximum productivity.

If one is doing initial process optimization design on a small scale system, which one intends to scale up, the above shows the importance of considering the large scale system properties. On a small scale system it is usually the column pressure rating that limits the achievable flow, but on a large scale system it is usually the pump’s capacity. Selection of packing particle size and mobile phase must be done with this in mind. Also notice that practical considerations, for example the response time for detectors and fraction collectors, must be taken into account as the equipment might prevent the use of the pumps max flow $F_{\mathrm{V,max,pump}}$ if this leads to very short cycle times, e.g. when using very short columns.

In this study we have always used the maximum achievable flow rate in the process optimizations and only determined the injection volume that gives the highest productivity. However, there are cases when one should consider also including the flow rate in the process optimization. For example when the compounds are very hard to separate (i.e., poor selectivity) and one cannot find a better column, when the yield requirements are very high (i.e., one wants almost base line separation) or when one has impurities that elute before or after the main compounds and must be completely separated from them.

Acknowledgment

This work was supported by the Swedish Research Council (VR) in the project grant “Fundamental studies on molecular interactions aimed at preparative separations and biospecific measurements”. The work was also supported by the Swedish Knowledge Foundation for the KK HÖG 2011 project “Improved Purification Processes to Satisfy Modern Drug Quality Assurance and Environmental Criteria” and by the Research Council for Environment, Agricultural Sciences and Spatial Planning for the project “High-Value Compounds from Agricultural and Forestry Waste by Sustainable Methods – an Interdisciplinary Approach for Bioresource Utilization”.

References

[1] G. Guiochon, D.G. Shirazi, A. Felinger, A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, 2nd ed., Academic Press, Boston, MA, 2006.

[2] K. Kaczmarski, J. Chromatogr. A 1176 (2007) 57.

[3] P. Forssén, R. Arnell, T. Fornstedt, J. Chromatogr. A 1216 (2009) 4719.

[4] J. Samuelsson, M. Enmark, P. Forssén, T. Fornstedt, Chem. Eng. Technol. 35 (2012) 149.

[5] P. Forssén, J. Samuelsson, T. Fornstedt, J. Chromatogr. A 1299 (2013) 58.

[6] G. Guiochon, Modeling for Preparative Chromatography, 1st ed, Academic Press, Amsterdam ; Boston, 2003.

[7] J.A. Jönsson, ed., Chromatographic Theory and Basic Principles, Dekker, New York, 1987.

[8] R.J. LeVeque, Finite Volume Methods for Hyperbolic Problems, Cambridge University Press, Cambridge ; New York, 2002.

[9] S. Javeed, S. Qamar, A. Seidel-Morgenstern, G. Warnecke, Comput. Chem. Eng. 35 (2011) 2294.

[10] M. Enmark, R. Arnell, P. Forssén, J. Samuelsson, K. Kaczmarski, T. Fornstedt, J. Chromatogr. A 1218 (2011) 662.

[11] J. Lagarias, J. Reeds, M. Wright, P. Wright, SIAM J. Optim. 9 (1998) 112.

[12] K.O. Bowman, L.R. Shenton, H.K.H. Lee, J.S. Chipman, Y. Wu, C.P. Tsokos, M. Fischer, J.M. Hilbe, B.C. Sutradhar, M.Z. Raqab, M.T. Madi, C.D. Lai, A.W. Kemp, S. Stević, S. Mase, O.S. Sharipov, G.E.P. Box, N. Wermuth, in:, M. Lovric (Ed.), Int. Encycl. Stat. Sci., Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, pp. 589–590.

[13] K. Kaczmarski, D. Antos, Acta Chromatogr. 17 (2006) 20.

[14] S. Golshan-Shirazi, G. Guiochon, J. Chromatogr. 506 (1990) 495.

Figure Captions

Figure 1: Number of sampled random systems and the corresponding width of the 95% confidence interval for the optimal productivity $P_{\mathrm{R,opt}}$ change median, for the first eluting compound, when ΔP_{max} increases 10%. The confidence interval was calculated from a kernel density estimate, with a normal kernel function, of the inverse cumulative distribution function.

Figure 2: Histogram of the optimal productivity $P_{\mathrm{R,opt}}$ change, for the last eluting compound, when the particle size d_{p} decreases 10%. The black line is a corresponding generalized hyperbolic distribution, estimated using a maximum likelihood procedure. The bar above the histogram shows the median and the 2.5 – 97.5 percentile range of the generalized hyperbolic distribution.

Figure 3: The bars shows the median and the 2.5 – 97.5 percentile range of the optimal productivity $P_{\mathrm{R,opt}}$ change when a system or adsorption property is changed 10%. Black bars are for the first eluting compound and grey bars are for the last eluting. The median and percentile ranges are calculated from the estimated generalized hyperbolic distribution.

Figure 4: The probability that a 10% change in a system or adsorption parameter will result in a change in the optimal productivity $P_{\mathrm{R,opt}}$ that exceeds a certain value, in (a) for the first eluting compound and in (b) for the last eluting compound. The probabilities are calculated from 1 - the cumulative distribution function of the estimated generalized hyperbolic distribution.

Figure 5: Optimal productivity $P_{\mathrm{R,opt}}$ change when a system or adsorption property is increased 10% from its current value, in (a) for particle size d_{p} increases, in (b) for efficiency N_{x} increase and in (c) for selectivity α increase. The total parameter range was divided into 15 subintervals and the symbols show, for each parameter subinterval, the median optimal productivity $P_{\mathrm{R,opt}}$ change when the parameter is increased 10%. The curves are two element power series fitted to the symbols.

Figure 6: Histogram of the optimal productivity $P_{\mathrm{R,opt}}$ change, for the first eluting compound, when the pump max flow $F_{\mathrm{V,max,pump}}$ is 2.4 L/min. In in (a) when the maximum column pressure drop ΔP_{max} is

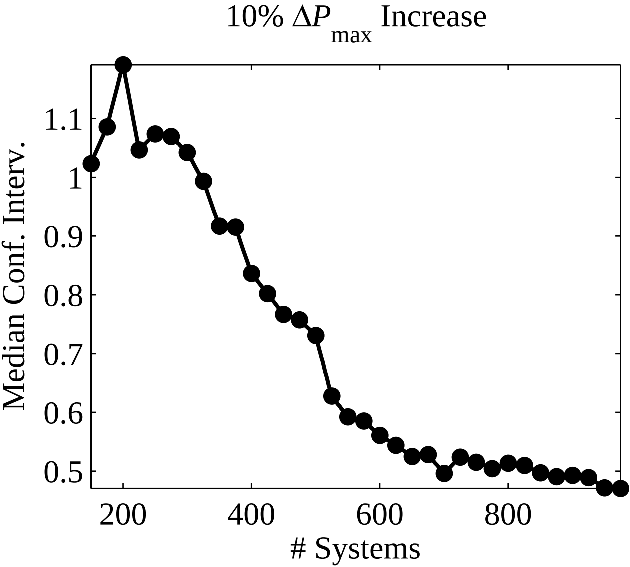
increased 10%, (b) when the column length L is decreased 10%, in (c) when the particle size d_p is increased 10% and in (d) when the mobile phase viscosity η is decreased 10%. The black line is the corresponding estimated generalized hyperbolic distributions and the gray lines are corresponding estimated generalized hyperbolic distributions, in (b) - (d) bimodal distributions, for systems without pump flow limitations.

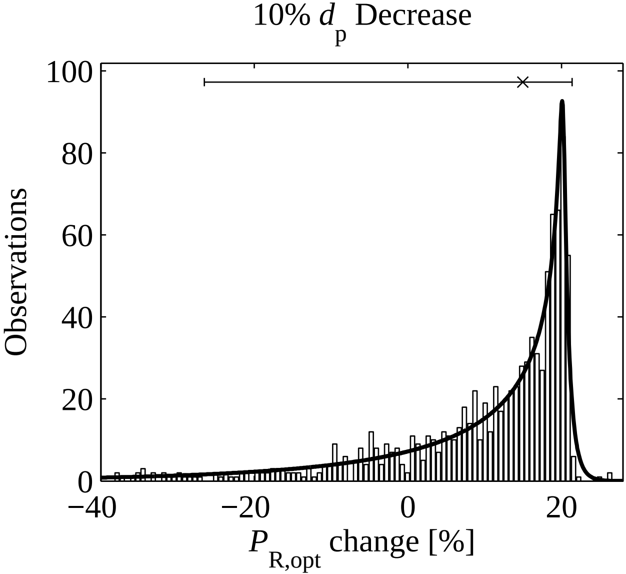
Table 1: Parameters values or intervals used to generate random systems if nothing else is stated. Notice that the Diffusion constant $D_{m,1}$ is related to the mobile phase viscosity η, the efficiency of the second component $N_{x,2}$ is related to that of the first $N_{x,1}$ (i.e., calculated from $D_{m,1}$ using Eq. (3)) and that the saturation capacities q_s are related to the corresponding sample concentration C_{samp}.

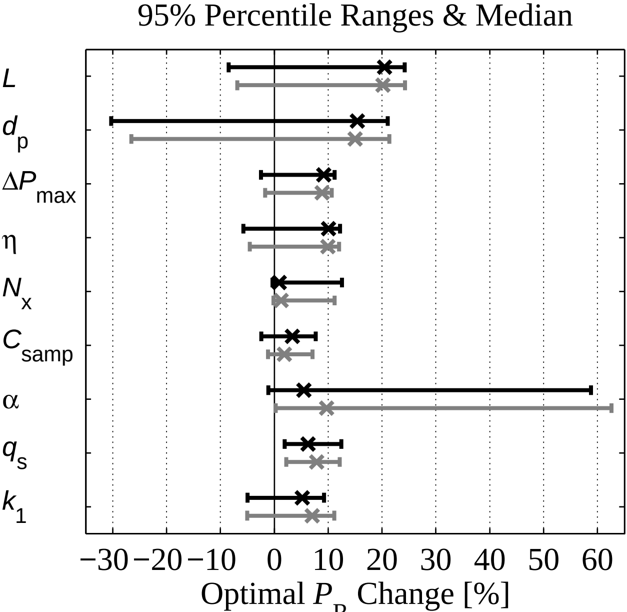
Parameter	Value
Column diameter, D	10 cm
Porosity, ε	0.76443
Column structural constant, θ	$6{\cdot}10^6$
Mobil phase concentration, C_{mob}	0.0 g/L
Pump max working pressure, $\Delta P_{max,pump}$	100.0 bar
Pump max flow, $F_{V,max,pump}$	∞ L/min
Column length, L	10 – 50 cm
Particle size, d_p	10 – 50 µm
Column pressure rating, $\Delta P_{max,col}$	40 – 100 bar
Mobile phase viscosity, η	0.3 – 2.3 cP
Diffusion constant 1, $D_{m,1}$	$1.390 \cdot 10^{-3}/\eta - 1.807{\cdot}10^{-3}/\eta$ cm^2/min
Theoretical plates 2, $N_{x,2}$	$0.9{\cdot}N_{x,1} - 1.1{\cdot}N_{x,1}$
Sample concentration 1, $C_{samp,\ 1}$	5.0 – 50.0 g/L
Sample concentration 2, $C_{samp,\ 2}$	5.0 – 50.0 g/L
Selectivity, α	1.2 – 3.2
Retention factor 1, k_1	0.5 – 5.0
Saturation capacity 1, $q_{s,1}$	$2{\cdot}C_{samp,1} - 30{\cdot}C_{samp,1}$
Saturation capacity 2, $q_{s,2}$	$2{\cdot}C_{samp,2} - 30{\cdot}C_{samp,2}$
Minimum required purity, PU_{min}	99 %
Minimum required yield, Y_{min}	0 – 98 %

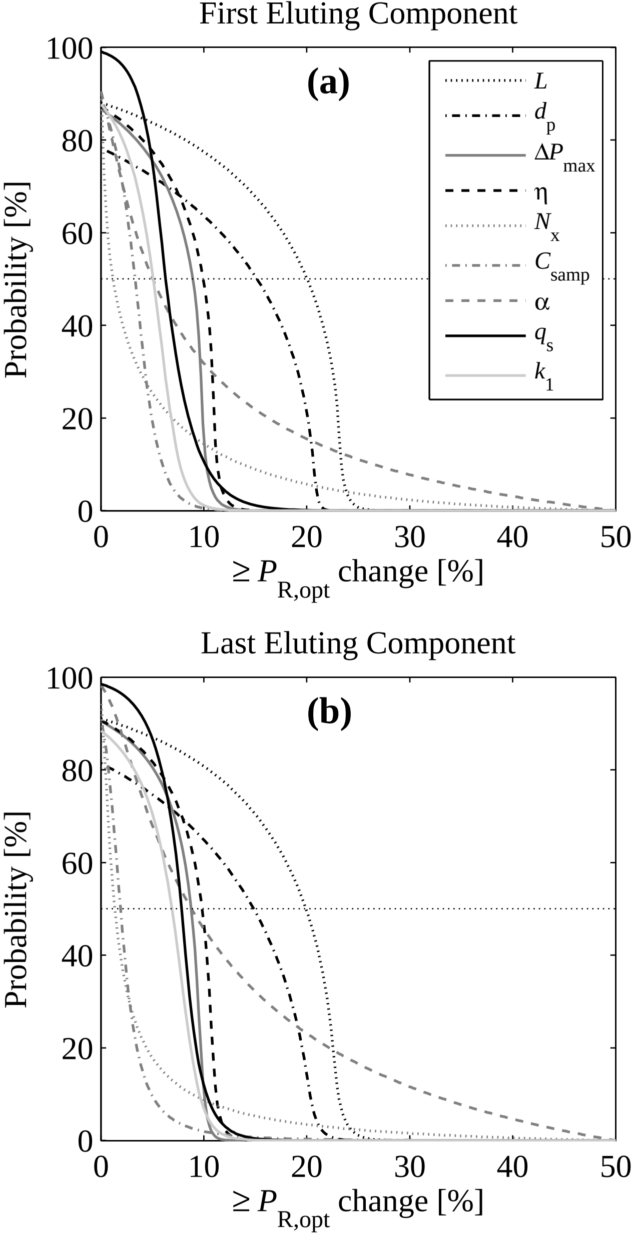
Table 2: Intervals for parameters that are calculated using the parameters in Table 1, see Eq. (1), Eq. (2) and Eq. (4).

Parameter	Interval
Maximum volumetric flow rate, $F_{V,max}$	0.1253 – 300.2 L/min
Maximum pressure drop, ΔP_{max}	40 – 100 bar
Linear velocity, u	2.087 – 5000 cm/min
Number of theoretical plates 1, $N_{x,1}$	50 – 20 806


10% $\Delta P_{\max}$ Increase
Median Conf. Interv.
1.1
1
0.9
0.8
0.7
0.6
0.5
200
400
600
800
Systems

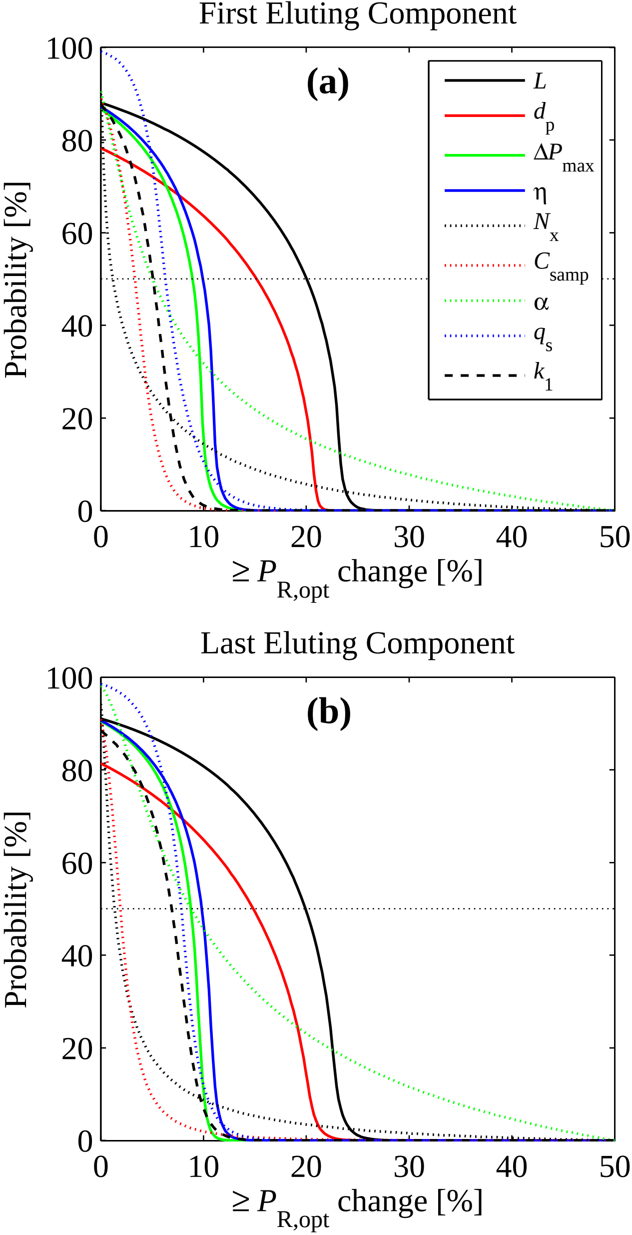

10% d_p Decrease
Observations
100
80
60
40
20
0
−40
−20
0
20
$P_{R,opt}$ change [%]

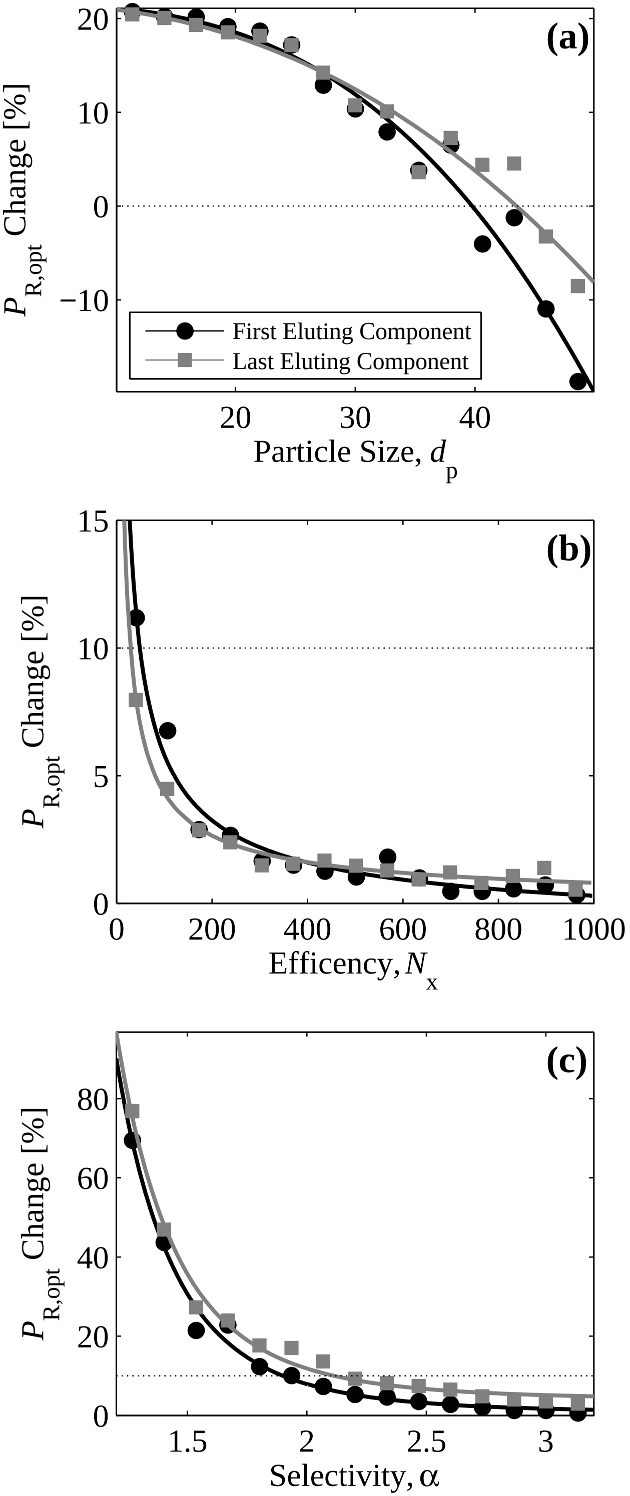

95% Percentile Ranges & Median
L
d_p
ΔP_max
η
N_x
C_samp
α
q_s
k_1
−30
−20
−10
0
10
20
30
40
50
60
Optimal P_R Change [%]

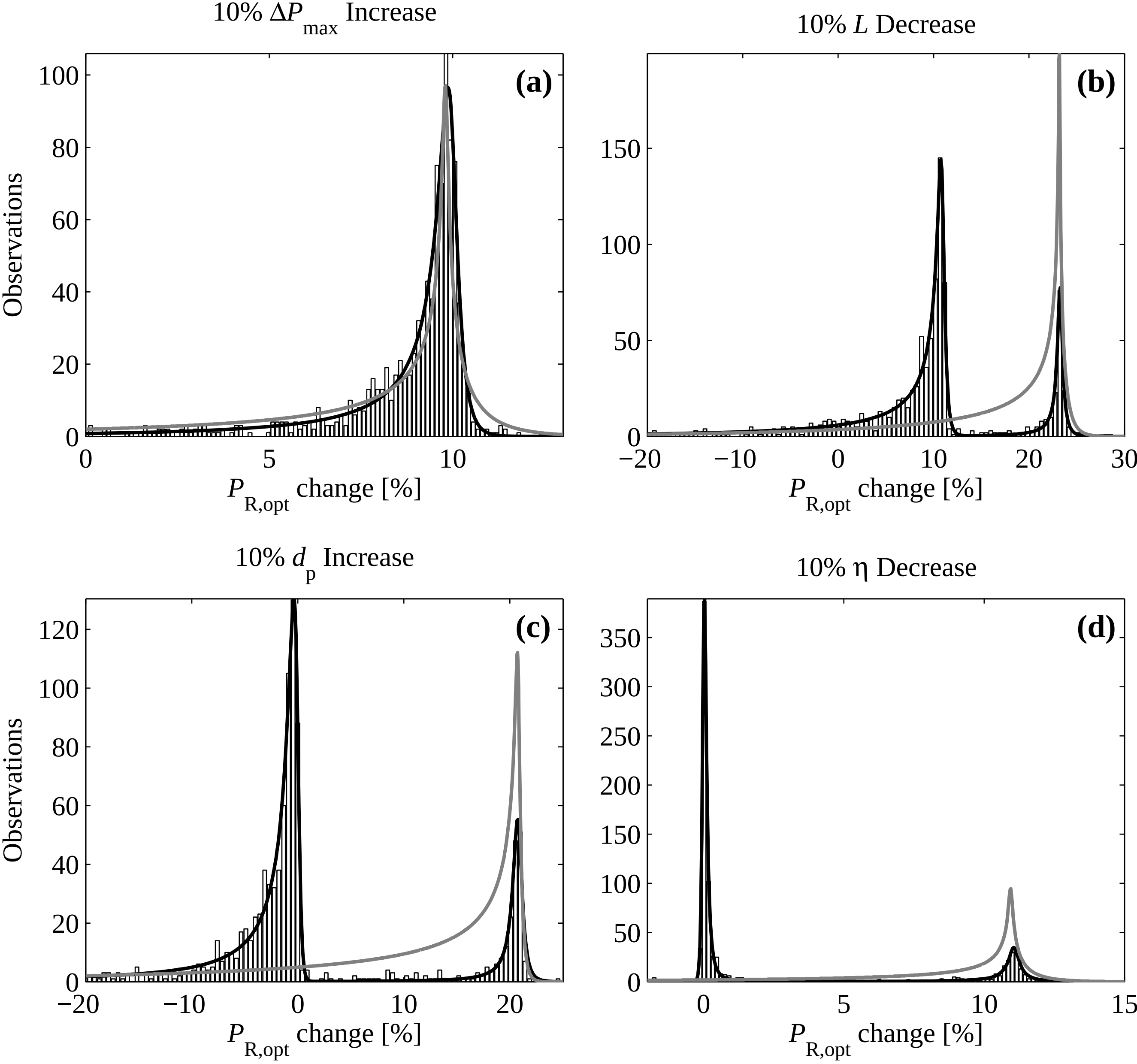


First Eluting Component

Last Eluting Component


First Eluting Component
(a)
Probability [%]
100
80
60
40
20
0
0
10
20
30
40
50
$\geq P_{R,opt}$ change [%]
L
d_p
ΔP_{max}
η
N_x
C_{samp}
α
q_s
k_1
Last Eluting Component
(b)
Probability [%]
100
80
60
40
20
0
0
10
20
30
40
50
$\geq P_{R,opt}$ change [%]


(a)
$P_{R,opt}$ Change [%]
20
10
0
−10
20
30
40
Particle Size, d_p
First Eluting Component
Last Eluting Component
(b)
$P_{R,opt}$ Change [%]
15
10
5
0
0
200
400
600
800
1000
Efficency, N_x
(c)
$P_{R,opt}$ Change [%]
80
60
40
20
0
1.5
2
2.5
3
Selectivity, α


10% ΔP_max Increase
(a)
Observations
0
20
40
60
80
100
0
5
10
P_R,opt change [%]
10% L Decrease
(b)
0
50
100
150
−20
−10
0
10
20
30
P_R,opt change [%]
10% d_p Increase
(c)
Observations
0
20
40
60
80
100
120
−20
−10
0
10
20
P_R,opt change [%]
10% η Decrease
(d)
0
50
100
150
200
250
300
350
0
5
10
15
P_R,opt change [%]